REMARKS

Applicants respectfully request reconsideration of the present application in view of the reasons that follow.

I. Status of the claims

No claims are added, canceled or amended in this paper. Claims 5 and 6 are pending and under examination.

II. Claim rejection – non-statutory, obviousness-type double patenting

Claims 5 and 6 stand rejected on the grounds of non-statutory obviousness-type double patenting over claims 1-9 of U.S. Patent No. 5,851,999 ("the '999 Patent"). The Office Action asserts that claims directed to a *pharmaceutical composition* comprising an expression vector encoding a truncated Flk-1 polypeptide and a pharmaceutically acceptable carrier renders obvious claims directed to *a cell line* comprising a recombinant vector encoding a truncated Flk-1 polypeptide. Applicants respectfully traverse this ground for rejection.

Without conceding to the correctness of the rejection and solely to expedite prosecution, a terminal disclaimer is filed herewith, thereby obviating the rejection with respect to the '999 Patent. Accordingly, reconsideration and withdrawal of the obviousness-type double patenting rejections in the present application is respectfully requested.

III. Claim rejection – 35 U.S.C. § 103(a)

Claims 5 and 6 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over U.S. Patent No. 5,185,438 ("Lemischka"), Matthews et al., *PNAS*, *88*:9026 (1991) ("Matthews"), and Terman et al., *BBRC*, *187*:1579 (1992) ("Terman"), in view of Ullrich et al., *Cell*, *61*:203 (1990) ("Ullrich"), and Ueno et al. (*Science*, *252*: 844 (1991) ("Ueno-1") and *JBC*, *267*:1470 (1992) ("Ueno-2")).

The Office Action asserts that because Flk-1 allegedly shares some aspects of homology to PDGFR, FGFR1 and EGFR, the "invention follows the teaching of the prior art, and achieves exactly the result that would be predicted on the basis of the prior art," and that "there are no unexpected results." Office Action at page 5. Thus, the Office Action alleges that not only would the skilled artisan find it obvious to make the claimed mutant polypeptide (amino acids 1-806 of SEQ ID NO: 2), but that it would not be surprising that the claimed mutant polypeptide would fold into a conformation that could dimerize with a wild-type Flk-1 polypeptide; that the dimer would bind VEGF; and that the dimer would prevent VEGF signaling. Applicants respectfully traverse this ground for rejection and assert that only with impermissible hindsight would the skilled artisan consider the claimed invention "obvious."

A. The cited references do not provide enough information about wild-type Flk-1 "classification" or function for the skilled artisan to predict Flk-1 mutant behavior

1. Lemischka

Lemischka describes Flk-1 and Flk-2 and references the Hanks article, which describes the presence of conserved amino acids in the catalytic domain of the family of receptor tyrosine kinases. Lemischka, however, states that Flk-2 is in a "new functional class" as compared to other receptor tyrosine kinases such as CSF and PDGF. Lemsichka then states that Flk-1 is not a member of the same class of receptors as Flk-2 based on expression in some more mature hematopoietic cells. Lemischka also does not state whether Flk-1 would be in the same class as CSF and PDGF. Thus, Lemischka does not provide any information whatsoever that Flk-1 would belong to the same class as other known receptor tyrosine kinases. Finally, Lemischka does not contain any suggestion directing the skilled person to the claimed truncated Flk-1 consisting of amino acids 1-806 of SEQ ID NO:2.

2. Matthews

Although the Office Action states that the primary references, including Matthews, teach the relatedness of Flk-1 to other receptor tyrosine kinases, Matthews actually points out differences between Flk-1 and other receptor tyrosine kinases. Specifically, Matthews describes Flk-1 as being considerably larger than other family members and that Flk-1 has 7 Ig-like domains, which is in contrast to the 5 Ig-like domains other tyrosine kinase possess.

Furthermore, Matthews states that while the kinase insert of Flk-1 contains potential sites for tyrosine kinase autophosphorylation, Flk-1 is distinct from c-kit, c-fms and Pdgfra/b because it lacks the consensus sequence for binding PI-3.

Matthews also states that "[i]nterestingly, Flk-1 appears to have seven immunoglobulin-like domains in its extracellular region, and together with Flt, may define an additional subfamily of receptor PTKs." This further highlights the differences between various receptor tyrosine kinases and underscores the inability to extrapolate Flk-1 function based on behavior of other receptor tyrosine kinases.

And lastly, Matthews does not contain any suggestion directing the skilled person to the claimed truncated Flk-1 consisting of amino acids 1-806 of SEQ ID NO:2.

3. Terman

Terman describes the KDR receptor and structural similarities of KDR to Flk-1. Terman, however, does not disclose the similarity between Flk-1 and a receptor tyrosine kinase that has been truncated in the secondary references, such as the FGF or PDGF receptor. In other words, because Terman does not talk about the structural similarity between Flk-1 and one of the receptors that has been truncated, this reference does not "teach the relatedness of Flk-1 to the receptors discloses by the secondary references" (Office Action dated 1-9-08 at page 5), as indicated by the Office as being the essence of the rejection.

Terman also does not contain any suggestion directing the skilled person to the claimed truncated Flk-1 consisting of amino acids 1-806 of SEQ ID NO:2.

4. Ueno I

Secondary reference Ueno I describes a PDGF receptor lacking most of its cytoplasmic domain that can form heterodimers with a wild-type PDGF receptor and block WT receptor function. Ueno I, however, states that other truncated receptor tyrosine kinases (such as the EGFR and insulin receptor) inhibited wild-type receptor function but "did not inhibit the autophosphorylation of wild-type receptors and the mechanisms by which the kinase-defective mutants inhibited wild-type receptor function were not known." Ueno I at 847. Thus, even receptors within the family of receptor tyrosine kinases can function differently and by different mechanisms, further supporting the inability to predict function based solely on structural similarities.

In other words, although the truncated PDGFR dimerized with a wild-type PDGF receptor and interfered with cell signaling, the mechanism by which this occurred was distinct from that of the EGF receptor and insulin receptor truncations. As such, the mechanism of inhibition of the truncated PDGF receptor cannot be extrapolated to a different truncated receptor tyrosine kinase. And similarly, a truncated PDGF receptor cannot predict the activity of other truncated receptor tyrosine kinases.

Moreover, Ueno I indicates that the truncated PDGF mutant lost its putative autophosphorylation sites (Ueno I at page 847, right col., 1st par.). In contrast thereto, the claimed truncated Flk-1 still contains a tyrosine residue, i.e. a putative autophosphorylation site in its cytosolic domain (amino acid 799 of SEQ ID NO:2). Thus, Ueno I would lead the skilled person away from the claimed truncated Flk-1.

5. Ueno II

Ueno II discloses a truncated FGF receptor but as provided above, the FGF receptor is structurally different from Flk-1. For example, the FGF receptor does not have the 7 Ig-like domains characteristic of Flk-1, KDR, and other more similar receptor tyrosine kinases.

Furthermore, Ueno II suggests that the wild-type FGFR and truncated FGFR heterodimers may activate the cytoplasmic signaling pathways differently than do the homodimers of the truncated FGFs. Accordingly, the mechanism of inhibition may again be different, even between truncated FGFs, depending on whether homo- or heterodimers are formed, which supports Applicants' position that structural similarity does not absolutely predict function.

Furthermore, Ueno II discloses that the truncated forms of FGFR1 used in this study did not have tyrosine residues in their cytosolic regions (Ueno II, p.1475, left col., 1st par. last four lines). In contrast thereto, the claimed truncated Flk-1 contains a tyrosine residue in the cytosolic domain, namely amino acid residue 799 of SEQ ID NO:2. Thus, Ueno II teaches away from the claimed truncated Flk-1 molecule.

6. Ullrich

Ullrich is simply a review article that describes EGF, PDGF, IGF-1 and CSF as a family of receptor tyrosine kinases but does not mention Flk-1 or its other more structurally similar receptor tyrosine kinases. As such, the combination of Ullrich with the primary references and/or the other secondary references does not teach or suggest each and every element of the claimed invention.

B. None of the cited references disclose that Flk-1 functions as a dimer; accordingly, activity of a Flk-1 mutant can not be predicted based on the activity of kinase receptor dimer mutants

The Office asserts that Terman teaches "that it would be desirable to investigate the dimeric combinations in which the receptor occurs; Terman does not doubt that the receptor is a

dimer." Office Action at page 4. Applicants respectfully disagree with the Examiner's characterizations regarding Terman. In fact, Terman states that *"[i]t is not known* whether KDR and *flt* can form functionally active dimers analogous to the PDGF receptor dimers" and "*it is not known* whether KDR, *flt* or heterodimer KDR/*flt* mediates mitogenic activity and/or vascular permeability." Terman at page 1585. Accordingly, the teaching of Terman does not substantiate the Examiner's assertions that Flk-1 was known to function as a dimer.

The Office also asserts that "[w]ith respect to Ullrich, combination of the subunit of SEQ ID NO: 2 in vivo in a cell that expresses Flk-1 would inherently result in a combination with a 'normal' subunit, regardless of whether the receptor were a homo- or hetero-dimer." Office Action at page 4. However, the claimed SEQ ID NO: 2 mutant is neither taught nor suggested in Ullrich. Accordingly, this argument is moot with respect to the claimed invention.

The Office Action also asserts that Ullrich teaches that "[r]eceptor oligomerization is a universal phenomenon among growth factor receptors." Office Action at page 4. Ullrich does indeed make this statement. However, this statement, alone or in combination with any or all of the cited references, does not in any way teach or suggest that the claimed mutant polypeptide would be able to "oligomerize."

The Office Action also cites Ullrich with respect to the function of various kinase mutants: "While the kinase activity of the various receptors was dispensable for their expression and targeting to the cell surface it was indispensable for signal transduction and induction of other early and delayed cellular responses...." Office Action at page 4. Based on this statement, the Office Action concludes "without knowing the subunit structure of the receptor, one would expect that a subunit lacking the tyrosine kinase domain would have dominant negative signaling effects." Office Action at page 5. Applicants respectfully disagree with the characterization of Ullrich and the conclusion reached. While Ullrich describes mutations of the EGF, insulin and PDF receptors, Ullrich does not teach or suggest Flk-1 or Flk-1 mutations. Moreover, Ullrich in combination with the cited references does not teach or suggest that wild-type Flk-1 functions as a dimer; accordingly, there is no reason one of skill in the art would conclude that the claimed

mutant Flk-1 would form a dimer with its wild-type counterpart. Further, one of ordinary skill in the art would not conclude that "without knowing the subunit structure of the receptor, one would expect that a subunit [of Flk-1] lacking the tyrosine kinase domain would have dominant negative signaling effects."

With respect to mutant dimerization and mutant-dimer function, the Office Action asserts that "Applicant are arguing limitations that are not found in the claims," and that "neither a dimer, nor any particular activity" is required in the claims. Office Action at page 5. Applicants respectfully traverse this assertion.

Because the ability of wild-type Flk-1 to dimerize was *unknown* at the time of filing, the fact that the claimed mutant Flk-1 could dimerize, that a dimer including the claimed mutant Flk-1 would bind VEGF, and that the a dimer including the claimed mutant Flk-1 would inhibit VEGF signaling, *is completely unexpected*.

C. Protein function cannot accurately be predicted based on structural homology

Assigning protein function based on sequence homology is viewed with skepticism in the art. For example, Wells illustrates that changes in amino acid sequence, even a change of a few amino acids, can result in proteins with unpredictable function (Wells, *Biochemistry*, *29*(37) 8509-17 (1990), EXHIBIT A). Moreover, Attwood (Attwood, *Science*, *290*: 471-473 (2000), EXHIBIT B) teaches that "[i]t is presumptuous to make functional assignments merely on the basis of some degree of similarity between sequences." Similarly, Skolnick et al. (Skolnick et al., *Trends in Biotech.*, *18*(1): 34-39 (2000), EXHIBIT C) teach that the skilled artisan is well aware that assigning functional activities for any particular protein or protein family based on sequence homology is inaccurate, in part because of the multifunctional nature of proteins (*see*

e.g., Skolnick et al. at Abstract and "sequence-based approaches to functional prediction," page 34).[1]

In the present situation, there is virtually no homology between the polypeptides at the amino acid level as evidenced by the three protein BLAST alignments provided below. The first alignment compares amino acids 1-806 of SEQ ID NO: 2 with PDGFR (human; Accession Number NP_002600); the second alignment compares amino acids 1-806 of SEQ ID NO: 2 with EGFR (human; Accession Number AAH94761), and the third alignment compares amino acids 1-806 of SEQ ID NO: 2 with FGFR1 (human; Accession Number AAH15035). The full alignment documents are provided as Exhibits D-F, respectively.

[1] MPEP § 2124 states that "in some circumstances a factual reference need not antedate the filing date," for example, when describing a "scientific truism." Both Attwood and Skolnick present such scientific truisms.

BLAST 1: alignment of amino acids 1-806 of SEQ ID NO: 2 with PDGFR

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Score = 62.8 bits (151),  Expect = 1e-13, Method: Compositional matrix adjust.
 Identities = 84/382 (21%), Positives = 153/382 (40%), Gaps = 49/382 (12%)

Query  41   LTILANTTLQITCRGQRDLDWLWPNAQRDSEERVLVTECGGGDSIFCKTLTIPRVVGNDT  100
            L +  ++T  +TC G   + W      +R S+E          D  F   LT+  + G DT
Sbjct  42   LVLNVSSTFVLTCSGSAPVVW-----ERMSQEPPQEM-AKAQDGTFSSVLTLTNLTGLDT  95

Query  101  GAYKCSYRD-----VDIASTVYVYVRDYRSPFIASVSDQHGIVYITENKNKTVVIPCRGS  155
            G Y C++ D       D    +Y++V D    F+ + +++   +++TE     + IPCR +
Sbjct  96   GEYFCTHNDSRGLETDERKRLYIFVPDPTVGFLPNDAEEL-FIFLTEITE--ITIPCRVT  152

Query  156  ISNLNVSLCARYPEKRFVPDGNRISWDSEIGFTLPSYMISYAGMVFCEAKINDETYQSIM  215
               L V+L  +  +          + +D + GF+       SY     C+  I D    S
Sbjct  153  DPQLVVTLHEKKGDVAL-----PVPYDHQRGFSGIFEDRSY----ICKTTIGDREVDSDA  203

Query  216  YIVVVVGYRIYDVILSPPHEIELSAGEKLVLNCTARTELNVGLDFTWHSPPSKSHHKKIV  275
            Y V  +      +V ++    + +  GE + L C       N  ++F W  P       +K
Sbjct  204  YYVYRLQVSSINVSVNAVQTV-VRQGENITLMCIVIG--NEVVNFEWTYP------RKES  254

Query  276  NRDVKPFPGTVAKM---FLSTLTIESVTKSDQGEYTCVASSGRMIKRNRTFVRVHT--KP  330
             R V+P    +  M     S L I S    D G YTC  +       ++  + +
Sbjct  255  GRLVEPVTDFLLDMPYHIRSILHIPSAELEDSGTYTCNVTESVNDHQDEKAINITVVESG  314

Query  331  FIAFGSGMKSLVEATVGSQVRIPVKYLSYPAPDIKWYRNGRPIESNYTMIVG--------  382
            ++     + +L  A +      + V + +YP P + W+++ R +  +     +
Sbjct  315  YVRLLGEVGTLQFAELHRSRTLQVVFEAYPPPTVLWFKDNRTLGDSSAGEIALSTRNVSE  374

Query  383  ----DELTIMEVTERDAGNYTV  400
                 ELT++ V   +AG+YT+
Sbjct  375  TRYVSELTLVRVKVAEAGHYTM  396
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BLAST 2: alignment of amino acids 1-806 of SEQ ID NO: 2 with EGFR:

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Score = 16.5 bits (31),  Expect = 7.7, Method: Compositional matrix adjust.
 Identities = 8/19 (42%), Positives = 8/19 (42%), Gaps = 0/19 (0%)

Query  472   PGQTSPYACKEWRHVEDFQ  490
              P Q  P A  E   V D Q
Sbjct  1071  PSQVLPPASPEGETVADLQ  1089
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BLAST 3: alignment of amino acids 1-806 of SEQ ID NO: 2 with FGFR1:

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Score = 57.8 bits (138),  Expect = 3e-12, Method: Compositional matrix adjust.
 Identities = 45/165 (27%), Positives = 72/165 (43%), Gaps = 28/165 (16%)

Query  634  DQGDYVCSAQDKKTKKRHCLVKQLIILERMA--PMITGNLE-NQTTTIGETIEVTCPASG  690
            D+G+Y C  +++      H    QL ++ER    P++   L  N+T  +G  +E  C
Sbjct  224  DKGNYTCIVENEYGSINHTY--QLDVVERSPHRPILQAGLPANKTVALGSNVEFMCKVYS  281

Query  691  NPTPHITWFKDNET--------------LVEDSGIVLRDGNRN-LTIRRVRKEDGGLYTC  735
            +P PHI W K  E                +++ +G+   D     L +R V  ED G YTC
Sbjct  282  DPQPHIQWLKHIEVNGSKIGPDNLPYVQILKTAGVNTTDKEMEVLHLRNVSFEDAGEYTC  341

Query  736  QACNVLGCARAET-LFIIEGAQEKTN-------LEVIILVGTAVI  772
              A N +G +     L ++E  +E+          LE+II    A +
Sbjct  342  LAGNSIGLSHHSAWLTVLEALEERPAVMTSPLYLEIIIYCTGAFL  386
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The alignments make clear that sequence homology between these polypeptides is essentially non-existent. Only small portions of SEQ ID NO: 2 can be aligned with PDGFR, FGFR1 or EGFR, and the resulting alignments have minimal amino acid identity.

Even in situations where there is some confidence of similar overall structure between two proteins, only experimental research can confirm the artisan's best guess as to the function of the structurally related proteins (*see e.g.*, Skolnick, in particular Abstract and Box 2). It is well known in the art that predicting protein function from sequence data is extremely complex. While it may be that one or more deletions are generally possible in any given protein, the positions within the protein's sequence where such deletions can be made with a reasonable expectation of producing a desired or expected function should be determined empirically for each protein. Certain positions within a sequence are critical to the protein's structure/function relationship, *e.g.*, such as various sties or regions directly involved in binding, activity and in providing the correct three-dimensional spatial orientation of binding and active sites (*see e.g.*, Wells).

Thus, given the aversion in the art to making functional assessments based on sequence homology in conjunction with the *complete lack* of sequence homology between amino acids 1-806 of SEQ ID NO: 2 and PDGR, FGFR1 and EGFR as shown above, there would be absolutely

no reason for the skilled artisan, at the time of filing, to assume or reasonably believe that the truncated form of Flk-1 would behave in a manner similar to a truncated form of PDGFR, EGFR or FGFR1. There is simply no ground for such an assumption.

D. Because *flt* was a known VEGF receptor with high affinity for VEGF, it was unexpected that the *flk-1* mutant would inhibit the cellular effects of VEGF binding

In addition, it was entirely unexpected that the truncated Flk-1 variant would have an inhibitory effect on the cellular response of VEGF. Such results were unexpected because at least one other receptor, *flt*, was known to bind VEGF with high affinity.[2] It also was known that *flt* is expressed in endothelial cells of a growing tumor.[3] Significantly, *flt* has a 50-fold higher affinity for VEGF than Flk-1.[4] Given the importance of VEGF signaling in angiogenesis during *inter alia*, development, wound healing and organ regeneration, some redundancy in the system would be expected. Consequently, the skilled artisan would not have expected that blocking the Flk-1 signaling pathway would shut down the cellular response to VEGF, resulting in suppression of angiogenesis and inhibition of tumor growth. Rather, one of ordinary skill in the art would have anticipated that the biological response to VEGF, such as the proliferation of blood vessels, would still be transduced through *flt* or some as yet undiscovered receptors.

E. Summary

The obviousness inquiry must rely on evidence available at the time of the invention. *See Eisai Co. Ltd. v. Dr. Reddy's Laboratories*, --F.3d--, 2008 WL 2791884 (Fed. Cir. 2008), citing *Takeda Chem. Indus. v. Alapharm Pty., Ltd.*, 492 F.3d 1350 at 1356 (Fed. Cir. 2007). And in 1992, the priority date of the present invention, Flk-1 had only just been identified (as evidenced

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[2] *See e.g.,* Terman et al., *BBRC, 187*:1579 (1992).

[3] *See*, Plate et al., *Nature, 359*: 845-848 (1992); Plate et al., *Cancer Research, 53*: 5822-5827 (1993). (Ref. A35)

[4] *See*, Waltenberger et al., *J. Biol. Chem., 269*: 26988-26995 (1994) (entered into the file via an IDS submitted 10-12-07).

by Lemischka) and the similarity between the VEGF and PDGF systems had not yet been characterized (Terman at 1585). Thus, the level of a person of ordinary skill in the field of Flk-1 receptor tyrosine kinases at the time of filing the present application was low.

Applicants respectfully assert that even in light of the combination of cited references, the skilled artisan would not have expected the claimed polypeptide – a deletion mutant of Flk-1 with very little amino acid homology to EGFR, FGFR or PDGFR, and not even known to function as a dimer – to be able to do the following:

(1) form a three-dimensional structure capable of dimerizing with a wild-type Flk-1 polypeptide,

(2) function with the wild-type polypeptide to bind VEGF, and

(3) to inhibit VEGF signaling function.

There would be no reason for the skilled and creative artisan to try to generate the claimed Flk-1 mutant, and there would be no reasonable expectation that such a mutant would even be functional, yet alone be beneficial. Applicants respectfully assert that the claimed cell lines are not obvious in light of the combination of cited references.

Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. § 103 is respectfully requested.

V. Conclusion

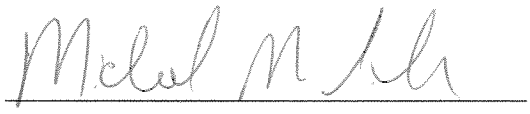
The present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by the credit card payment instructions in EFS-Web being incorrect or absent, resulting in a rejected or incorrect credit card transaction, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicants hereby petition for such extension under 37 C.F.R. §1.136 and authorize payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

Date: June 16, 2010

By 

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